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Detecting food borne pathogens using electrochemical biosensors: An overview

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Abstract
Food safety is becoming increasingly an important public health issue, as food borne diseases present a widespread and growing public health problem in both developed and developing countries. The rapid and precise monitoring and detection of food borne pathogens are some of the most effective ways to control and prevent human food borne infections. Biosensors offer rapid and cost effective method of food borne pathogen detection. It utilizes the unique properties of biological and physical materials to recognize a target molecule and effect transduction of an electronic signal. Varieties of biosensors have been developed, viz., electrochemical (amperometric, potentiometric and impedance), optical (light scattering, fibre optics and SPR) and mass based (piezoelectric and surface acoustic). This article gives an overview of electrochemical biosensors for detection of pathogenic bacteria in the food industry. Electrochemical biosensors have some advantages over other analytical transducing systems, such as the possibility to operate in turbid media, comparable instrumental sensitivity, and possibility of miniaturisation. Basically electrochemical biosensor can be based on potentiometric, amperometric or impedimetric/conductimetric transducers. In recent years, nanotechnology has emerged as a promising field for solving food safety issues in terms of detecting contaminants. The introduction of nanomaterials into electrochemical sensors makes them suitable to reach lower detection limit, higher sensitivity values and bring novel labelling opportunities including multi detection capabilities.

Keywords: Food, pathogens, electrochemical ,biosensors

Introduction
Food-borne pathogens are very diverse in their nature and keep causing major public health problems worldwide. Pathogens micro-organisms that cause diseases are transmitted by various food items. Therefore food safety and emerging food biosecurity issues are important to consumers, food manufactures and food producers. The increased movements of humans across the globe and globalization of food trade has lead to changes in the dietary habits and changes in the patterns of food production consumption and distribution. These changes have led to improved nutritional health and at the same time these changes have also thrown up new food safety challenges (Sankarankutty, 2014) [39]. Food laboratory analysis has several key challenges that hinder the proper detection of pathogens which include: uneven distribution of bacteria in food; presence of indigenous microbes; and the heterogeneous nature of food matrices (Mandal *et al*., 2010) [29]. Other important factors to consider in food analysis are time and sensitivity of the analyses, and to minimize human errors and labour costs. Conventional methods for pathogen detection are time-consuming, laborious, lack sensitivity and specificity, and required specialized equipment and trained users (Fournier *et al*., 2013) [16]. The development of rapid and reliable detection methods for food-borne pathogens is in progress. Examples of these methods include biosensors, immunological methods, and nucleic acid based assays (Mandal *et al*., 2010) [29]. Immunological or nucleic acid-based methods needs extensive sample preparation and are not amenable to miniaturization for on-site detection. Biosensors offer a rapid and cost-effective method of bacterial detection which can be performed at the point of care without the need for a specialist user. Biosensors can be used in various fields like in food (pathogen, additives), environmental monitoring, clinical diagnoses and biodefense due to their high sensitivity, selectivity and quick response (Thakur and Ragavan, 2013) [45]. In recent years, nanotechnology has emerged as a promising field for solving food safety issues in terms of detecting contaminants. The sensing ability of the biosensor detection systems is being improved by using of nanomaterials such as magnetic nano-particles (MNPs), carbon nanotubes (CNTs), nanorods (NRs), quantum dots (QDs),

nanowires (NWs), nanochannels (NCs), etc. Use of nanomaterials into electrochemical sensors makes them more suitable to detect lower detection limit, higher sensitivity and multidetection capabilities.

Biosensor

A biosensor is an analytical device by which an analyte is detected by bioreceptors with a physicochemical component (Hasan *et al*., 2014) [18]. The first biosensor was coined by Clark and Lyons in 1962, known as father of biosensor concept. They developed an oxygen electrode combined with enzyme glucose oxidase to measure dissolved oxygen in blood (Sharma *et al*., 2013) [41]. Biosensor comprises three fundamental components: a bioreceptor or biorecognition element, which recognise the desire analyte; a transducer, which convert biological signal into electrical signal and a signal processing system to present these electrical signals into an appropriate form as presented into fig: 1.

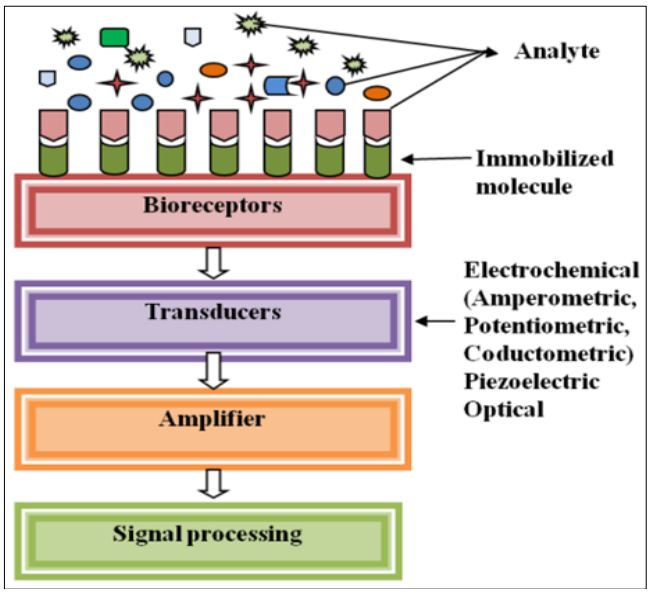

Fig 1: Basic components of Biosensors

Bioelement is immobilized on sensor surface by one of the following immobilization techniques: physical adsorption, covalent binding, matrix entrapment, inter molecular cross-linking and membrane entrapment (Fig. 2). The physical adsorption of bioreceptor depends on Vander Waals and hydrophobic forces, hydrogen bonds, and ionic forces. Polymeric membranes used for adsorption of these bioelement on sensor surface are cellulose, collodion, silica gel, glass, hydroxyapatite and collagen. This method is simple, cost-effective, however, the forces employed are not very strong and biomolecules attached may be released or not persist. In covalent binding sensor surface is modified so that the biological materials can be attached to it. The functional group that may take part in this binding are amino group, sulfhydryl group, hydroxyl group, phenolic group, thiol group etc. This type of binding is generally used for enzyme immobilization. In enzymatic biosensors functional groups which are not essential in the enzyme is utilized for binding. This method improves distribution of the bioelements, as well as reproducibility and homogeneity of the surfaces. Matrix entrapment employ the entrapment of biomolecules which are not direcly attached with the polymeric gel matrix but get trapped. Polymeric material such as polyacrylamide, starch, alginate, pectate, polyvinyl alcohol, polyvinyl chloride, polycarbonate, polyacrylamide, cellulose acetate and silica gel are often used. Cross-linking involves intermolecular cross-linking of biomolecules or analyte in the presence/absence of solid support. Bi-functional or multi-functional cross linking reagents such as glutaraldehyde, hexamethylene di-isocyanate, 1,5-difluoro 2,4-dinitrobenzene and bisdiazobenzidine-2, 2'-disulphonic acid, etc., are used. The most commonly used cross-linking agent in this type of biosensor is glutaraldehyde. Encapsulation is enclosing of biomolecules or analyte within the semi-permeable polymeric membranes and helps in attachment of it to the sensor surface. Biomolecules immobilized by this process are very stable (Monošík *et al*., 2012; Mohanty and Kougianos, 2006) [34, 33].

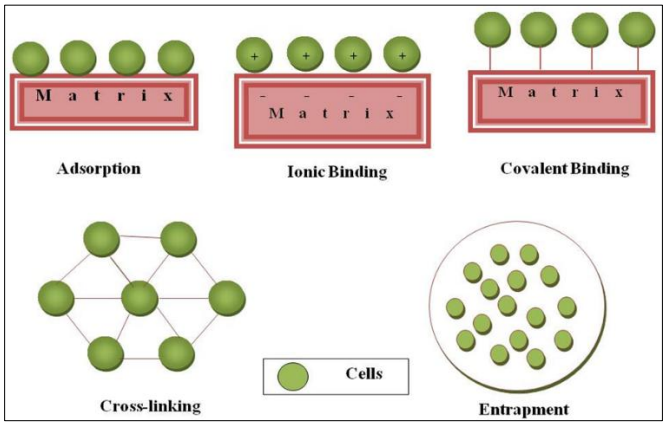

Fig 2: Immobilization techniques for bioelement

Classification of biosensors

Biosensors can be classified into different types according to mechanism of biological selectivity (biosensing component) or on basis of the transducer components as described below.

Bioreceptors or Biosensing Components

A bioreceptor is molecule species which utilizes biochemical mechanism for detection. The bioreceptors can be categories into two types: catalytic type (enzymes, cells or tissues) and affinity type (antibodies, nucleic acid).

Enzyme bioreceptor

The enzymes used as bioreceptor are of oxidase type that can react selectively with specific analyte, consume dissolved O_2, and produce H_2O_2 that is an easily detectable compound (Hasan *et al*., 2014) [18]. The most commonly used enzyme is horseradish peroxidase and beta-galactosidase. The detection of pathogenic bacteria in food can be done by labelling the antibodies with these enzymes. The advantages of this bioreceptor are the commercial availability of enzymes, high purity, specificity, suitability for different transduction techniques, detects a wide range of analytes (Sharma *et al*., 2013) [41].

Cells as bioreceptor

These sensors can use bacteria, yeast or higher eukaryotic cells including vertebrate or mammalian cells. The advantages of living cells as recognition element are: sensitivity, they present functional analysis for biochemical agents, very low detection limit (Velusamy *et al*., 2010) [49]. Banerjee and Bhunia, 2010 [6] developed cell based biosensor for rapid detection of multiple pathogenic bacteria (*Listeria monocytogenes*, enterotoxigenic *Bacillus*, *Vibrio*, *Micrococcus* and *Serratia*) and toxin (from *Staphylococcus aureus*, *Clostridium perfringens*, *Stoichactis helianthus*, *L. monocytogenes*, and *Bacillus*) in food safety application.

Antibody bioreceptor

Antibodies are universal bioreceptors used in biosensors. They are proteins produced by B-Lymphocytes in response to antigenic stimulation. An antigen-specific antibody fits its unique antigen and makes it as an analytical tool for detection of the chemicals, biomolecules, microorganisms etc (Sharma *et al*., 2013) [41].

Nucleic acid biosensor

This type of biosensors utilizes DNA, RNA and peptide nucleic acid as a bioreceptor and gaining popularity because of their high sensitivity and selectivity due to strong base pair affinity (Borgmann *et al*., 2011) [7]. Several researches has been done on nucleic acid based biosensor for detection of food borne pathogen like *E. coli*, *Salmonella* spp., *C. jejuni* etc. (Sharma *et al*., 2013) [41].

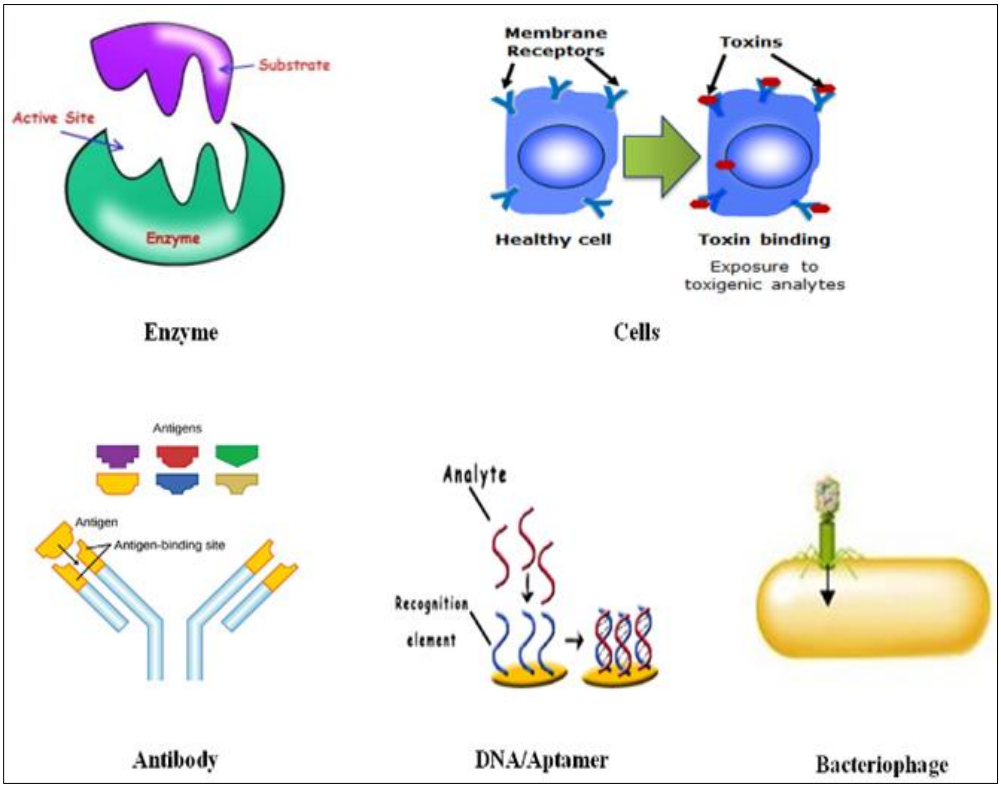

Fig 3: bioreceptors

Bacteriophage bioreceptor

Phages are bacterial viruses of 20-200 nm in size that bind to target bacteria through specific receptors present at the surface of host cells and inject their genetic material. These viruses recognize target bacteria through receptors located on their tail. They are capable of targeting specific bacteria make them attractive candidates for use as probes in sensor devices (Shabani *et al*., 2015) [40]. Phages is used as biorecognition component for the exposure of various pathogens such as *E. coli*, *Staphylococcus aureus* and *Bacillus anthracis* spores by adopting diverse sensing platforms.

Transducer component

The transducer plays an important part in biosensor. Four major types of transducers are used in biosensor devices are electrochemical, calorimetric (thermometric), mass (piezoelectric or surface acoustic wave devices) and optical.

Electrochemical transducers

Electrochemical devices usually monitor the current at a fixed voltage (amperometry), the voltage at zero current (potentiometry), or measure conductivity or impedance changes.

Optical transducers

Optical transducers are capable to sense minute changes in the refractive index or thickness when microorganisms bind to receptors immobilized on the transducer surface. Optical biosensors are divided into two categories: - labelled system (Fluorescence, Luminescence, Transmission and Scattering) or label free system (Ellipsometry, Surface Plasmon resonance, Resonant mirror sensor, Interferometer) (Mutlu, 2011) [35].

Mass based transducers

The mass-sensitive sensors exploit piezoelectric crystals for pathogen detection. This type of transducer measures the change in frequency through a piezoelectric material as bacterial binding occur to receptor bind to transducer (Shabani *et al.*, 2015) [40].

Electrochemical biosensors

Electrochemical biosensors are achieving popularity over other biosensors is owing to its low cost, good sensitivity and selectivity, use in turbid media, and miniaturization potential for food-borne pathogens detection. (Palchetti and Mascini, 2008) [37]. Electrochemical biosensors utilizes chemical reaction involving immobilized biomolecules and target analyte which influence measurable electrical properties of solution such as an electric current or potential by producing or consuming ions (Zhang *et al*., 2008) [58]. These are classified into amperometric, potentiometric, impedimetric and thermometric which depend on the type of transducer used (Lazcka *et al*., 2007) [23].

Amperometric biosensors: Amperometric transduction is universal electrochemical biosensor for pathogen detection (Sharma *et al*., 2013) [41]. This biosensor measures the quantity of current produced at a constant potential between a working electrode and a reference electrode. (Zhang *et al*., 2008) [58]. Typical equipment for amperometric measurement includes a three electrode cell, based on a working electrode, a reference electrode and an auxiliary electrode; as well as the voltage source and a device for measuring current and voltage (Mutlu, 2011) [35]. An example of an amperometric apparatus is presented in Figure 4. The simplest amperometric biosensors make use of Clarks oxygen electrode, which determines the quantity of oxygen (present in the analyte) reduced. It typically relies on an enzyme system that catalytically converts analyte into product that can be oxidized or reduced at a working electrode. Horse radish peroxidase (HRP) and alkaline phosphatise (AP) are amongst the commonly used enzymes (Zourob *et al*., 2008) [59]. Examples of pathogen detection by amperometric biosensor are

Microbial metabolism based biosensor

Metabolic processes in microorganism in which specific marker enzyme particularly of oxido-reductase produced is used for detection purpose. Different types of microbial metabolism based biosensors have been reported in the literature. This biosensor is mostly used for water samples examination to identify coliform by their metabolic product released enzyme β-D-glucuronide, glucuronosohydrolase (GUS) and β-d-galactosidase (Arora *et al*., 2013) [5]. Conventional methods of *E. coli* detection using GUS enzymes or β-GAL are lengthy and time consuming. To over edge this time-consuming protocol, Togo *et al*., 2007 [6] developed an efficient method for GUS detection using bacteria-based biosensors by immobilization of *Moraxella* species. They resulted that conversion of p-nitrophenyl-b-D-glucuronide(metabolic product of GUS enzyme of E. Coli) to p-nitrophenol (PNP) and D-glucuronic acid by *Moraxella* indicates the presence of *E. coli*. Neufeld *et al*. (2003) [36] developed an amperometric electrochemical biosensor for quantification of coliform *E. coli* K-12 using bacteriophage immobilized on screen-printed carbon electrodes. Bacteriophages invade the bacteria and results in release of the intracellular bacterial enzyme of β-D-galactosidase from them. Enzymatic activity was measured amperometrically using *p*-aminophenyl-*â*-D-galactopyranoside (*â*- PAPG) as substrate (0.8 mg/mL). The sensitivity of this sensor is 1 CFU/100 ml of sample.

Immunosensors

Detection of micro-organism using antibodies is another successful technology utilized in Immunosensors. In immunologic detection antibodies are immobilized on electrode surface or magnetic beads. laczka *et al*. (2011) [22] developed immunosensor for the detection of *E. coli* in a microfluidic system coupled with immunomagnetic beads. The specific antibody attached to magnetic particles was suspended on top of a gold electrode surface in the interior of a flow cell through magnetic force. The bacterial sample was poured into the cell, followed by the addition of a horseradish peroxidase (HRP)-conjugated antibody label which binds in a sandwich manner. Detection is through the electrochemical monitoring of the activity of horseradish peroxidase (HRP), an enzyme label, through its catalysis of hydrogen peroxide (H_2O_2) in the presence of the mediator hydroquinone (HQ). Developed a gold nanoparticle-labeled biosensor for quick and sensitive recognition of *E. coli* O157: H7. Gold nanoparticles (AuNP) were attached with polyclonal antibodies and then introduced to magnetic nanoparticle (MNP)-target to form a sandwitch MNP-target-AuNP and produce the signals. The lower limit of detection is at 10^1 cfu/ml with a dynamic range of 10^1 to 10^6 cfu/ml. Similarly Lu *et al*., 2016 developed an amperometric immunosensor by immobilization of HRP-labeled antibody against *Listeria monocytogenes* onto the surface of the novel multiwalled carbon nanotube fibers. Detection of *L. monocytogenes* was judge by alteration of direct electrochemical signal caused by the binding of antigen and antibody. This developed immunosensor is tested for milk sample inoculated with different concentration of *L. monocytogenes.* The lower limit of detection for *L. monocytogenes* is 1.07×10^2 cfu/ml with a dynamic range of 10^2 to 10^5 cfu/ml.

DNA based biosensors

In recent years a variety of electrochemical DNA sensors have been developed for recognition of the bacterial nucleic acid. A DNA biosensor is defined as an analytical device incorporating an oligonucleotide, with a known sequence of bases, either integrated within or intimately associated with the electrode (Zourob *et al*., 2008) [59]. Nowadays DNA screen-printed microarrays coated with different probes have been developed for simultaneous detection of multiple DNA-target sequences (Teles *et al*., 2008) [44]. Fernandes *et al*., 2014 [1] developed an amperometric nanoparticle based genosensors for the simultaneous multiple detection of the gene of *E. coli* O157:H7 and the nuc gene of *S. aureus.* This genosensors chiefly composed of nanotracers (such as PbS and CdS), fixing probe (fDNA), target DNA (tDNA) and detective probe (dDNA). The dDNA was complementary sequence of another end of the tDNA, which binds with the nanoparticle tracers (NTs), such as lead sulfite (PbS) and cadmium sulfite (CdS), that act as a signal reporter and amplifier. The stripping signals of Pb and Cd have a linear relationship with the logarithmic concentrations of tDNAs. The signal increases with increasing logarithmical concentration of tDNA. The detection limits of this DNA sensor are as low as 1×10^{-12} M of the nuc gene of *S. aureus* using CdS, and 1×10^{-12} M of the gene of *E. coli* O157:H7 using PbS NTs. This DNA based electrochemical biosensor provides high specificity and selectivity in detection of target DNA.

Potentiometric biosensors

Potentiometric biosensing utilizes the ionselective electrodes to measure the potential of a solution based on specific interactions with ions in the solution. This method measures the electrical potential difference between working electrode and reference electrode. The reference electrode is one whose potential remains invariant during the entire duration of measurement. The working electrode undergoes significant change in its potential even for small changes in the analyte concentration (Ahmed *et al*., 2014) [2]. These are amongst the least common biosensors utilized for pathogen detection. Nowadays, semi-conductor based physico-chemical transducers are more common. ISFETs and LAPS (light addressable Potentiometric sensor) based systems especially are convenient for biosensor construction (Zhang *et al*., 2008) [58]. A LAPS is made up of a semiconductor chip (n-type silicon), covered with a silicon-dioxide insulating layer, placed in contact with the sample solution. The potential that results from the different charge distributions that exist at the insulating layer/solution interface and the

semiconductor/insulator interface is directly influenced by the binding interactions occurring at the probe-modified insulating layer surface, and the signal is enhanced by illumination with a modulated light beam (Marco *et al*., 1996). Ercole *et al*., 2003 have demonstrated detection of *E. coli* in vegetable foods (lettuce, sliced carrots, and rucola) by using Light Addressable Potentiomentric Sensor. The presence of *E. coli* was detecting by pH variations due to NH3 production by an urease–*E. coli* antibody conjugate. Vegetable samples were washed with peptone water at pH 6.8 blended either in a stomacher or in a sonicator, to detach bacterial cells and to recover them in the liquid medium. This liquid phase was analyzed by potentiometric alternating biosensing (PAB) system. This method for detection of food borne pathogen is fast, sensitive and concentration of up to 10 cells/ml were detected in an assay time of ca. 1.5 h. Zelada *et al*., 2010 developed real time potentiometric biosensor made up of carbon nanotubes chemically linked to aptamer as probes to selectively detect *E. coli* in complex liquid samples such as milk and fruit juice. Biosensors exposed to increasing concentration of *E. coli* in sample and EMF was measured. The real-time EMF bacterial binding generated a linear signal with increasing concentration, with a detection limit of as low as 6 colony forming units/mL (CFU) in complex matrices such as milk or 26 CFU/mL in apple juice. The biosensor can be easily built and used, is regenerated without difficulty, and can be used at least five times with no loss in the minimum amount of detected bacteria. Similarly Zelada *et al*., 2012 developed real time potentiometric biosensor for detection of staphylococcus aureus in pig skin contaminated with target microorganism. Electromotive force (EMF) was measured in a single-wall carbon nanotube- based chemically linked to aptamer system. Limit of detection for S. aureus was 8×10^2 cells/ml when the aptamer was covalently bound to the nanotubes and 10^7 cells/ml when bound non-covalently.

Impedimetric biosensors

The impedance is one of the earliest physicochemical methods that has been utilized for detection and quantification of microorganism. The first impedance method for detection of bacteria was described by G.N. Stewart in 1899. Impedimetric biosensors, also known as capacitive biosensors, measure the change in conductance of the medium resulting from microbial growth or microbial metabolism of inert substrates into electrically charged ionic compounds (Sharma *et al*., 2013) [41]. Impedimetric biosensor for detection of microorganism can be classified into two types depending on the presence or absence of specific bio-recognition elements. The first type works by measuring the impedance change caused by binding of targets to bioreceptors (antibodies and nucleic acids) immobilized onto the electrode surface, while the detection principle of the second type is based on metabolites produced by bacterial cells as a result of growth. Main advantages of these biosensors are the unrestricted measurement of the molecule of interest, with no requirements for the analyte to be an enzymatic substrate or for formation of electroactive species as in amperometric sensing (Ahmed *et al*., 2014) [2], capabilities to monitor large number of samples simultaneously and a relatively short detection time (between 6 to 24 hrs) (Hayes, 1992) [19]. Disadvantages of these types of biosensors are variable reproducibility, high limits of detection, and problems with nonspecific binding. However, with continued improvements and the advancement of miniaturization of equipment, has improved the impedimetric biosensor for microbial detection.

Mechanisms for Impedance detection of microorganisms

Based on these electrical-related properties of bacterial cells, three mechanisms have been reported for developing impedance-based methods for detection/quantification of bacterial cells:

1. Detection based on bacterial metabolism: This approach is based on the measurement of changes in electrical impedance of a culture medium or a reaction solution resulting from the bacterial growth. The impedance changes are mainly caused by the release of ionic metabolites from live cells into the medium, which includes main energy metabolism (catabolism) and minor ions (such as K+, Na+ ion channels). The impedance change is usually measured over time using a pair of electrodes submerged in the growth medium or the reactant solution. The measured electrical signals are then graphically plotted on the ordinate against the incubation times on the abscissa, producing impedance growth curves. The shape of the impedance growth curve well reflects the bacterial growth phases (yang *et al*., 2008) [55]. Gomez *et al*., 2005 studied the impedance based microbial detection(*L. monocytogen*) using silicon based microfluidic biochips where cells are collected and concentrated from a liquid sample and their growth and metabolism is detected as they are incubated with a volume 400 pL inside the chip. Concentration and capture of microbial sample is obtained by the use of dielectrophoresis on the bacterial cells and metabolism detection is achieved by means of impedance measurements. They also demonstrated that this technique significantly reduce the time needed to detect the presence of the bacteria.

2. Detection based on the insulating properties of the cell membrane: Bacterial cells consist of adjacent structures that have very different electrical properties. The bacterial cell membrane consists of a lipid bilayer and numerous proteins that are primarily responsible for transport of ions, nutrients, and waste across the membrane. This membrane is general highly insulated with its conductivity around 10–7 S/m. In this type of impedimetric biosensor, bioreceptors (such as antibodies, nucleic acids, bacteriophages and lectins) are immobilized on electrode surface which selectively bind to particular bacteria. Because of highly insulating cell membrane, bacterial cells attached to an electrode surface can effectively reduce the electrode area that the current reaches and thus increase the interface impedance (yang *et al*., 2008) [55].

Malvano *et al*., 2016 [28] developed a disposable screen printed carbon electrodes (SPCE) immunosensor based on electrochemical impedance spectroscopy detection of ochratoxin A in food samples. The anti-OTA was immobilized on gold modified SPCE surface through a cysteamine layer. The change in electrical properties (capacitance) of the electrode surface is measured using different concentration of ochratoxin A. The limit of detection for ochratoxin A is 0.25 ng/mL in many food samples. The developed immunosensor has very low detection limit, high sensitivity, simplicity and its results are suitable for fast OTA measurement in food matrices. Similarly Moghtadar *et al.*, 2016 used pencil graphite electrode deposited by gold nanorods for impedimetric detection of E. coli K12 cells. Bacteriophages were adsorbed on these gold nanorod

electrodes. The value of interfacial charge-transfer resistance values (R_{ct}) increases as E. coli binds with bacteriophage, reaches maximum in about 25-30 min and then decreases as bacterial lysis occur due to phage invasion on the electrode surface. In contrast, there was no time dependent changes with the non-target bacteria- *S. aureus* (no infection no lytic activity). This method for bacteria detection is very simple and inexpensive with a limit of detection of 10^3 CFU/mL in about 100 µL bacterial suspension.

3. Detection based on the release of ionic cytoplasm substances: Interior of bacterial cell contains DNA, nutrient storage granules, ribosomes, many dissolved charged molecules which make inside of it highly conductive (1 S/m). Cell lysis or release of intracellular ions to a low-conductance medium can change the impedance. This change in impedance is used for detection of bacterial cell.

Some commercially available systems such as the Bactometer (bioMerieux), Malthus ATanalyzer (Malthus Instruments), Bac Trac TM are used for pathogen monitoring and quality assurance purposes (table 2).

Table 1: Examples of electrochemical biosensors for food borne pathogen analysis

Organism	Method	Electrode	Detection Limit	Sample	Reference	Bioreceptor
E. coli	Potentiometric aptamer-based biosensor		6 colony forming units/mL (CFU) in complex matrices such as milk or 26 CFU/mL in apple juice	Apple juice, milk	Gustavo *et al.* (2010)	DNA
Sulfate-reducing bacteria	Potentiometric	Glassy carbon electrode	2.3 _ 10–2.3 _ 107 CFU/ml		Wan *et al*., 2010	None
Staphylococcus aureus	Potentiometric	Single-walled carbon Nanotubes	8 _ 102 CFU/m	Pig skin	Zelada *et al*., 2012	Aptamer
Staphylococcus Aureus	Potentiometric	Carbon nanotube aptamer based electrode	Single CFU/mL		Hernandez *et al*., 2014; Hernandez *et al*., 2012; Cao *et al*., 2009	DNA Aptamers
E. coli K-12	Amperometric	Screen-printed carbon Electrodes	1 CFU/100 ml		Neufeld *et al*., 2003 [36]	Bacteriophage
Heat-killed *E. Coli*	Amperometric	saturated calomel electrode	3 _ 101–3.2 _ 106 CFU/ml, down to 15 CFU/ml		Li *et al*., 2013	Biotinyl Antibody
Staphylococcus aureus	Amperometric	DropSens screen-printed gold electrodes	1 CFU/ml	Milk	De Avila *et al*., 2012	Antibody
E. coli	Amperometric	Photolithographic gold	55 cells/ml in PBS, 100 cells/ml in milk	Phosphate buffer solution, milk	Laczka *et al*., 2011 [22]	Antibody
Streptococcus agalactiae	Amperometric	Screen-printed carbon Electrodes	10CFU/ml	PBS	Vásquez *et al*., 2016 [48]	Antibody
L. monocytogen	Amperometric	Gold nanoparticle modified screen printed carbon electrode	2 log CFU/g	Blueberry	Davis *et al*., 2013	Antibody
Salmonella pullorum	Amperometric biosensor	screen printed electrode (SPE) modified gold nanoparticle-coated reduced graphene oxide	100CFU mL-1	Food sample	Fei *et al*., 2016	Antibody
Salmonella typhimurium	Amperometric detection	Gold electrode	10 CFU/ml	Skim and whole milk	Alexander *et al*., 2016	Polyclonal antibody
Staphylococcus Aureus	Cycli voltametry	antibiotic modified carbon paste electrode	-	Bacterial sample	Amlil *et al*., 2016 [4]	-
Bacillus anthracis	Cyclic voltammetry	Gold modified screen printed electrode	10pM	Liquid sample	Raveendran *et al*., 2016 [38]	SS-DNA
S. aureus	Cyclic voltammetry, Electrochemical Impedance Spectroscopy		1.23 ng/ml	Beef	Abdalhai *et al*., 2014 [1]	SS-DNA
Escherichia coli O157:H7and *Salmonella typhimurium*	impedimetric immunosensor	screen- printed interdigitated microelectrode	2.05×10^3CFU/g and 1.04×10^3 CFU/ml	E. coli O157:H7 in ground beef and S. Typhi-murium in chicken carcass rinse water	Xu *et al*., 2016	Antibody
Escherichia coli O157:H7	Impedimetric detection		100 CFU/mL	PBS	Wan *et al*., 2016	Antibody
E. coli O157:H7	Impedimetric detection	alumina nanoporous membrane with hyaluronic acid	83.7 CFU/mL	Whole milk	Joung *et al*., 2013 [21]	Antibody
E. coli O157:H7	Impedimetric	Gold nanoparticles	1.5 × 104 and 1.5 ×	Ground beef and	Wang *et al*., 2013	Antibody

	detection	modified graphene paper electrode	103 CFU/mL in ground beef and cucumber, respectively	cucumber		
E. coli	Impedance spectroscopy		1 bacterial/ul	Water	Mannoor *et al*., 2010	Antimicrobial peptide
E. coli	Impedimetric detection	Gold deposited graphite electrode	10^3 CFU/ml	Bacterial suspension	Moghtada *et al*., 2016	Bacteriophage
Gram +ve bacteria, selectively *L. monocytogen*	Impedimetric detection	interdigitated gold microelectrodes	10^3 CFU/ml	Milk	Etayash *et al*., 2014 [14]	Antimicrobial peptide
Listeria innocua	Impedimetric detection	gold screen printed electrode (SPE)	105 CFU/mL	Milk	Tolba *et al*., 2012 [47]	Bacteriophage endolycin
S. Typhimurium	Impedimetric detection	gold nanoparticles and poly(amidoamine)-multiwalled carbon nanotubes	103 CFU/mL	Fat free milk	Dong *et al*., 2013 [12]	Antibody
Listeria monocytogenes	Impedimetric detection	platinum interdigitated array microelectrodes	5.39 ± 0.21 CFU/mL	-	Sidhu *et al*., 2016 [42]	DNA Aptamer

Table 2: Commercially available electrochemical biosensors (The names of commercial products/companies used in this study are for information purpose only {Sharma *et al*., 2013) [41]

Manufacturer	Instrument	Measured property
Malthus Inc., Stoke-on-Trent, UK	Malthus 2000	Potentiometric, conductometric, field Effect
Biosensori SpA, Milan, Italy	Midas Pro	Amperometric
Bactomatic Inc., Princeton, USA	Bactometer	Impedimetric
Biomerieux, France	Bactometer	Impedimetric
Sy-Lab, Austria	Bac Trac	Impedimetric
Malthus Instruments	Malthus ATanalyzer	Impedimetric

Conclusions

As the world becomes more concerned about the impact of food on human health, the safety against biowar and the demand for rapid detection techniques has increased commercially. Conventional methods for food borne pathogens identification and detection are sensitive, time-consuming and laborious. Biosensors- based technologies are increasingly being used to detect foodborne pathogen as an alternative to conventional methods. It offer rapid, real time and multiple analyses from food samples. There are multiple biosensors that simultaneously detect multiple analyses with minimum interferences. Much of work has been done in the field of electrochemical biosensors for food safety. Electrochemical biosensor offer lower manufacturing costs and ease of system miniaturization and integration, with impedance spectroscopy becoming increasingly popular due to the lack of reagents and ability to detect any analyte without the need for electroactive species. In spite of having developed a number of biosensors for detecting food-borne pathogens, it is still a challenge to create biosensors for the reliable and effective determination of microorganisms in real food samples.

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